Estimation of nitrogen concentration and *in vitro* dry matter digestibility of herbage of warm-season grass pastures from canopy hyperspectral reflectance measurements

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Abstract

Remote sensing of nitrogen (N) concentration and *in vitro* dry matter digestibility (IVDMD) in herbage can help livestock managers make timely decisions for adjusting stocking rate and managing pastures during the grazing season. Traditional laboratory analyses of N and IVDMD are time-consuming and costly. Non-destructive measurements of canopy hyperspectral reflectance of pasture may provide a rapid and inexpensive means of estimating these measures of nutritive value. Using a portable spectroradiometer, canopy reflectance was measured in eight warm-season grass pastures in the USA in June and July in 2002 and 2003 to develop and validate algorithms for estimating N concentration and IVDMD of herbage. Nitrogen concentration of herbage was linearly correlated ($r = 0{\cdot}82$; $P < 0{\cdot}001$) with a ratio of reflectance in the 705- and 1685-nm wavebands (R_{705}/R_{1685}) and IVDMD was correlated with R_{705}/R_{535} (r = 0·74; $P < 0{\cdot}001$). Compared with simple linear regressions of N concentration and IVDMD in herbage with two-waveband reflectance ratios, multiple regression, using maximum r^2 improvement, band-depth analysis with step-wise regression, and partial least-squares regression enhanced the correlation between N concentration and IVDMD of herbage and canopy reflectance values ($0{\cdot}81 \le |r| \le 0{\cdot}90$; $P < 0{\cdot}001$). Validation of the prediction equations indicated that multiple regression only slightly improved accuracy of a model for predicting N concentration and IVDMD of herbage compared with simple linear regression of reflectance ratios. Results suggest that the N concentration and IVDMD of herbage of warm-season grass pastures can be rapidly and non-destructively estimated during the grazing season using canopy reflectance in a few narrow wavebands.

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Introduction

Measurements of nitrogen (N) concentration and *in vitro* dry matter digestibility (IVDMD) are important for determining the nutritive value of pasture and hay crops in the management of pastures and livestock (Ball *et al.*, 2001). Accurate and timely estimation of the N concentration and IVDMD of pasture during the grazing season can help livestock managers make appropriate decisions concerning fertilizer application, stocking rate and the feeding of supplements. Laboratory Kjeldahl and combustion methods have long been used for determining the N and crude protein (N × 6·25) concentrations of herbage or forage (Adesogan *et al.*, 2000). Determining IVDMD with extracted rumen fluid is a commonly used laboratory method. These laboratory methods are accurate and reliable, but usually time-consuming and costly.

Real-time reflectance measurements at the fresh leaf or canopy scale in the field offer an alternative for estimating the chemical composition of plants, such as N and chlorophyll concentrations of leaves (Yoder and Pettigrew-Crosby, 1995; Sims and Gamon, 2002; Zhao *et al.*, 2005a,b). Using spectral data from a 224 waveband (370–2500 nm region) Airborne Visible ⁄ Infrared Imaging Spectrometer (AVIRIS) instrument, Serrano *et al.* (2002) developed two indices for assessing N and lignin concentrations in Mediterranean vegetation. Studies suggest that remotely sensed data at the leaf, canopy or landscape level could be used to monitor plant physiology and chemistry (Chappelle *et al.*, 1992; Curran *et al.*, 1992; Peñuelas and Filella, 1998; Daughtry *et al.*, 2000; Peñuelas and Inoue, 2000; Goel *et al.*, 2003) and nutrient status (Read *et al.*, 2002; Starks *et al.*, 2004). If N concentration and IVDMD of herbage can be predicted by non-destructive measurements of canopy reflectance in a few wavebands via a spectroradiometer, it would reduce laborious field sampling and sample processing and would provide timely information for managing pastures and livestock.

Herbage quality varies as a function of precipitation, temperature, light, nutrient availability, plant maturity and management which affect ground cover, chemical composition of the plant and leaf structure (Campbell, 1996; Peñuelas and Filella, 1998; Kokaly and Clark, 1999; Fales and Fritz, 2007). Changes in variables, such as N concentration and IVDMD, therefore, should affect properties of canopy reflectance in a wide range of spectra from 400 to 1695 nm. Richardson *et al.* (1983) investigated relationships between values of canopy reflectance in red and near-infrared wavebands and N concentration of 'Alicia' hybrid bermudagrass and concluded that remote sensing could be a useful tool for rangeland management. Everitt *et al.* (1985) found leaf reflectance at 500 and 550 nm was highly correlated with N concentration of leaves in buffelgrass (*Cenchrus ciliaris* L.). More recently, Lamb *et al.* (2002) reported that leaf reflectance in the edge of the red waveband (690–740 nm) could be used to estimate both leaf N concentration and total N concentration of ryegrass (*Lolium perenne* L.) pasture. Zhao *et al.* (2005b) reported that the N concentration of leaves of sorghum (*Sorghum bicolor* L.) was also related to leaf reflectance in the same range and to a simple two-waveband reflectance ratio. Because the plant canopy of pasture grasses mainly consists of foliage, N concentration in above-ground biomass or the total N content of the biomass should be correlated with canopy reflectance. In a recent study, Mutanga *et al.* (2004) found that concentrations of N, P, K, Ca and Mg in grass pastures could be predicted using continuum-removed absorption features of measurements of canopy reflectance.

Starks *et al.* (2004) used a modified partial least-squares (PLS) regression to determine relationships between pasture canopy reflectance in 252 narrow wavebands (ranging from 368 to 1100 nm) and neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and N concentrations of herbage in bermudagrass (*Cynodon dactylon*, L) pastures. They found that the N concentration of herbage could be predicted using canopy reflectance in the 252 wavebands, with r^2 values ranging from 0·63 to 0·76 for the different measurements of nutritive value. Even though concentrations of NDF, ADF and N of herbage were estimated using canopy reflectance in hundreds of narrow wavebands (Starks *et al.*, 2004), determination of the most important wavebands for estimating the nutritive value of herbage was not part of their study. Furthermore, they did not investigate relationships between canopy reflectance and IVDMD of herbage.

In this study, the relationship between N concentration and canopy reflectance is further explored. Unlike the study of Starks *et al.* (2004), a limited number of wavebands or combination of wavebands is identified that may be used to construct prediction algorithms. This analysis is extended to include IVDMD of herbage because the literature describing the relationships between canopy reflectance and IVDMD is limited. Moreover, IVDMD is a direct measure of digestibility whereas ADF concentration is used to indirectly estimate digestibility, which is considered to be the least accurate of existing methods (Ball *et al.*, 2001).

Both N concentration and IVDMD of bermudagrass have their highest values in the early part of the grazing season which then decline as the herbage senesces. The relationship between N concentration and IVDMD, however, is weak. For example, Riday and Brummer (2006) found that only about 0·19 of the variability in N concentration of several sub-species of alfalfa (*Medicago sativa*) was associated with IVDMD values. Kamalak *et al.* (2004) found a small positive relationship (r^2 = 0·28) between N concentration and IVDMD in the leaves of shrubs and small trees grazed by small ruminants. A linear regression analysis of IVDMD and N concentration of herbage from several cultivars of bermudagrass found an r^2 value of only 0·25 (P. J. Starks, unpublished data).

To determine relationships between canopy reflectance and N concentration and IVDMD of herbage, a 2-year experiment was conducted in eight well-established warm-season grass pastures in central Oklahoma, USA. The specific objectives of the study were to: (i) develop reflectance algorithms for N concentration and IVDMD in herbage using different statistical methods and (ii) validate the algorithms and compare the accuracy of the different models for prediction *in situ* of these two measures of nutritive value.

Materials and methods

Experimental location

The study was conducted in eight warm-season bermudagrass (*Cynodon dactylon*, L.) pastures at the USDA-ARS Grazinglands Research Laboratory (Lat. 35°32′N, Long. 98°02′W), El Reno, OK, USA in June and July of

2002 and 2003. Four of the pastures were monocultures of Common bermudagrass (a commonly used perennial of livestock production systems in mid-south and south-east USA), which had an area of 1·2 ha, while the other four pastures consisted of approximately 0·70 of Common bermudagrass and 0·30 of other warm-season grasses, and which were 1·6 ha. All the pastures were established in 1991 or 1992 and had a similar soil type [Brewer silt clay loam (fine-loamy, mixed, thermic Udic Rhodustalfs)] and previous management. The monocultures were grazed by sheep (12 sheep ha^{-1}), while the mixed pastures were grazed by beef cattle at a stocking rate of three cattle ha^{-1} in both years. The pastures received one application of 67·3 kg urea-N ha^{-1} in early May of both years. Livestock were introduced to the pastures in early June of both years and continuously grazed the pastures during the experiment.

Measurements

Prior to taking measurements of canopy reflectance, each pasture was stratified into eight subplots (0·15–0·25 ha) to maximize the probability of capturing spatial variability extant in each pasture. Each stratum was numbered one to eight, for the purposes of assigning the subplot spectra to either the calibration or validation data set. Canopy hyperspectral reflectance was measured in the 350–2500 nm wavelength range on clear days between 10:00 and 12:30 h Central US Standard Time from early June to late July using a portable ASD FieldSpec FR spectroradiometer ([1]Analytical Spectral Devices Inc., Boulder, CO, USA).

Canopy reflectance was measured at a randomly selected site in each subplot within each pasture, being careful to avoid any site which would not be grazed by livestock (e.g. bare spots and manure piles). The optical head of the spectroradiometer was mounted on a boom 2 m above and perpendicular to the ground surface. The radiometer had a 25° field-of-view (FOV), producing an area of view with a diameter of 0·87 m. After the ASD instrument was optimized, reflectance of a white Spectralon (Labsphere, Inc., Sutton, NH, USA) panel, as a reference, was measured prior to taking three measurements of canopy reflectance at each sampling site. The data on canopy reflectance were expressed as relative values by dividing them by reflectance readings of the white reference panel.

In 2002, after canopy reflectance was measured at each sampling site, all above-ground vegetation in a 0·25-m^2 area within the ASD FOV was immediately clipped to a height of about 1 cm above the soil surface. In 2003, because of double the number of fields being sampled than in 2002, only about half of the strata were sampled in a given field on a given date. All strata within a field, however, were sampled at some time during the study. The herbage samples were transported to a laboratory and dried at 65°C for 48 h in a forced-air oven, weighed and ground through a 2-mm screen in a laboratory mill. Nitrogen concentration of the ground samples was determined in duplicate using an automated combustion instrument (LECO, St Joseph, MI, USA). IVDMD was determined using a digestion with rumen fluid incubated at 39°C for 48 h according to the procedure of Goering and Van Soest (1970).

Analysis of data

The maximum, minimum, mean, standard deviation and coefficient of variation were calculated from the laboratory measurements of N concentration and IVDMD. Additionally, mean herbage mass of dry matter (DM) was determined for each field on each sampling date.

Reflectance values in three ranges (i.e. 350–400, 1350–1450 and 1700–2500 nm) were first omitted from the reflectance data sets because of instrument noise or location of these wavebands within regions of high atmospheric moisture absorption. The remaining reflectance data were averaged over 10-nm intervals to decrease the amount of data for analysis, giving a total of 120 wavebands between 400 and 1700 nm. The three canopy reflectance spectra measured at each sampling site were averaged to yield four to eight spectra per pasture per sampling date. Based on the pasture stratification described above, spectra from the odd-numbered strata were placed in the calibration data set for algorithm development while spectra from the even-numbered strata were placed in the validation data set for algorithm testing. The combination of pastures (four in 2002 and eight in 2003), sampling sites (four to eight per pasture), sampling dates (seven in 2002 and nine in 2003), and years of study (two) led to a total of 264 spectra available for analysis, with n = 132 for the calibration data set and n = 132 for the validation data set.

Nitrogen concentrations associated with the spectra in the calibration data set were paired with their nearest neighbour, based on experimental field and sampling date, in the validation data set and a linear regression performed. The results from the linear regression revealed that about 0·43 (r^2 = 0·43) of the variation in the calibration data set was associated with the variability in the validation set. This low r^2 value suggests

[1]Mention of specific trade or product names does not imply endorsement or preferential treatment by USDA-ARS to the exclusion of any other product that may be suitable.

that the robustness of algorithms developed from the calibration data set could be fairly tested with the validation data set.

To develop prediction algorithms, first a linear regression was performed to select the wavebands where reflectance values had the greatest r^2 values with N concentration and IVDMD of herbage. The reflectance values at these selected wavebands were used as the numerators and reflectance values at all other wavebands were used as denominators to calculate reflectance ratios, according to Zhao *et al.* (2005a). The r^2 values of N concentration and IVDMD of herbage with the reflectance ratios were further determined using SAS PROC CORR (SAS, 1997).

In multiple regression analysis, especially for hyperspectral remote-sensing data, the collinearity or the co-dependence of various variables is a major concern. Under such situations, the method of maximum r^2 improvement (MAXR) is recommended (Yu, 2000) and has been used for hyperspectral reflectance data analysis (Goel *et al.*, 2003). To minimize the risk of over-fitting because of spectral variability that is independent of the chemical composition of leaves, Kokaly and Clark (1999) used band-depth analysis with step-wise regression to determine the chemical composition of leaves. Partial least-squares (PLS) regression is also a commonly-used method for predicting the chemical composition of plant tissue (Starks *et al.*, 2004). To find the best functional relationships between the measured N concentration or IVDMD of herbage and canopy reflectance in narrow wavebands, four different regression approaches were used: (i) simple linear regression of N concentration and IVDMD of herbage with the two-band reflectance ratio that has the greatest r^2 with the given variable of nutritive value; (ii) multiple regression using the MAXR with a total of five-waveband entrances; (iii) band-depth analysis and step-wise multiple linear regression (Kokaly and Clark, 1999; Mutanga *et al.*, 2004) and (iv) PLS regression with all the 120 wavebands (Starks *et al.*, 2004). When performing simple linear or multiple regression analyses for development of models and their associated regression coefficients, reflectance ratios or reflectance values recorded in various wavebands were considered as independent variables (x), and N concentration or IVDMD of herbage were the dependent variables (y).

Nitrogen concentration and IVDMD of herbage in the validation data set were predicted based on the algorithms developed from the calibration data set and on the reflectance values of the validation data set. Predicted N concentration and IVDMD values were plotted against laboratory measurements to evaluate performance of the prediction algorithms. The root-mean-square error of prediction (RMSEP) was calculated according to the following equation to determine the precision of estimation (prediction errors) between the measured and predicted values:

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2}{n}}$$

where $\hat{y}_i$ is the predicted value for N concentration or IVDMD, y_i is the measured value, and n is the number of samples. Goodness of fit between predicted and measured N concentration and IVDMD of herbage was evaluated using model efficiency (ME) defined by Nash and Sutcliffe (1970) as:

$$\mathrm{ME} = 1 - \sum (Y_{\mathrm{measured}} - Y_{\mathrm{predicted}})^2 / \sum (Y_{\mathrm{measured}} - Y_{\mathrm{mean}})^2$$

where Y_{measured} is the measured value for N concentration or IVDMD, $Y_{\mathrm{predicted}}$ is the predicted value and Y_{mean} is the mean of measured values for N concentration or IVDMD. The ME can range from 1 to $-\infty$. If ME = 1, the model accurately estimates the measured data, ME = 0 implies that the mean of the measured data is as good an overall predictor as the model, while a negative ME value indicates that the measured mean is a better predictor than the model.

Results

Herbage mass, N concentration and *in vitro* dry matter digestibility of herbage, and canopy spectra

Herbage mass varied greatly between fields and over time within fields (Figure 1). Field 8 produced more herbage mass on a given date than the other fields. On the sampling dates of 24 June and 8 July in 2002, field 8 produced 1950 kg DM ha^{-1} more herbage mass than field 6 (the least productive of the four fields). In 2003, fields 5, 6, 7 and 8 produced 1327 kg DM ha^{-1} less herbage mass than in 2002. Fields 1–4 were not sampled in 2002 but in 2003 these fields produced 890 kg DM ha^{-1} more herbage mass, on average, than fields 5–8.

Nitrogen concentration of herbage had a greater coefficient of variation (CV; 27·5%) than IVDMD (CV; 16·3%). Nitrogen concentration of herbage ranged from 6·1 to 24·8 g kg^{-1} DM with a mean of 14·7 g kg^{-1} DM and IVDMD values ranged from 0·398 to 0·946 with a mean of 0·614 across years, pastures, measuring dates and sampling sites (Table 1).

Patterns of canopy hyperspectral reflectance were similar between 2002 and 2003, but differences in canopy reflectance among individual samples, collected from different pastures, sampling sites and times, within a year were clearly detected (Figure 2a). Furthermore,

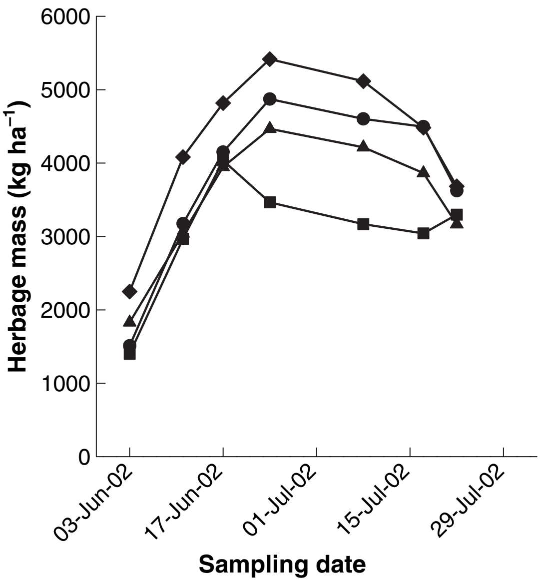

Figure 1 Herbage mass (kg DM ha^{-1}) of fields 5 (●), 6 (■), 7 (▲) and 8 (◆) during 2002.

canopy reflectance values centred at 675 nm had the greatest CV values across the samples among all the 120 wavebands from 400 to 1695 nm in both years (Figure 2b).

Development of algorithm

The r^2 values between N concentration and IVDMD of herbage with canopy reflectance depended on the wavebands used in the linear regression of the data in the calibration data set (Figure 3a). Overall, the r^2 values of IVDMD with reflectance were lower than those of N concentration with reflectance in most wavebands. The reflectance centred at 700–710 nm (i.e. R_{705}) had the greatest r^2 with both N concentration and IVDMD of herbage (Figure 3a). Although reflectance values in most wavebands were significantly correlated with these two variables ($P < 0{\cdot}05$), reflectance in a single narrow waveband having the greatest r^2 value, could only explain 0·55 of variation in N concentration and 0·40 in IVDMD (Figure 3a).

Reflectance ratios using 705 nm in most other wavebands improved the linear relationships (Figure 3b) between canopy reflectance and N concentration and IVDMD of herbage, i.e. greater r^2 values than simple reflectance. The two-waveband reflectance ratios having the greatest r^2 values with N concentration and IVDMD were R_{705}/R_{1685} ($r^2 = 0{\cdot}672$) and R_{705}/R_{535} ($r^2 = 0{\cdot}552$) respectively (Figure 3b).

Scatter plots indicated that relationships between forage N and R_{705}/R_{1685} and between IVDMD and R_{705}/R_{535} could be described in a linear fashion (graphs not shown). The linear equations and r^2 values, derived from the calibration data set, are given in Table 2. Based on the multiple linear regression analysis using MAXR, the r^2 values of the relationship of N concentration and IVDMD of herbage with canopy reflectance increased as the number of wavebands increased (Figure 4). Reflectance values in the first three to five selected wavebands had the highest correlations with the variation in N concentration and IVDMD. For instance, when wavebands in the model increased from one to five, the r^2 value increased from 0·55 to 0·80 for N concentration and from 0·40 to 0·65 for IVDMD. Further increase in wavebands yielded a negligible increase in r^2 value for N concentration and only slightly increased the r^2 value for IVDMD (Figure 4). The regression, using MAXR, of reflectance with N concentration and IVDMD indicated that the five most important wavebands were 675, 705, 1495, 1535 and 1585 nm for N concentration and 505, 705, 1165, 1655 and 1695 nm for IVDMD (Table 2). Equations developed from regressions, using MAXR, with entrance of the first five most important wavebands for N concentration and IVDMD are given in Table 2. Compared with the two-band reflectance ratio models, multiple regressions using MAXR increased the r^2 value for the relationship between N concentration and IVDMD of herbage and canopy reflectance. Similarly, step-wise regression with band depth analysis also yielded greater r^2 values than the two-band reflectance ratios (Table 2). PLS regression incorporating all 120 wavebands provided little to

Table 1 Values of maximum, minimum, mean, standard deviations of mean (s.d.), and coefficient of variation (CV) for nitrogen (N) concentration and *in vitro* dry matter digestibility (IVDMD) of herbage of warm-season grass pastures across fields, measuring dates, sampling sites and years (n = 264).

Variable	Maximum	Minimum	Mean	s.d.	CV (%)
N concentration (g kg^{-1} DM)	24·8	6·1	14·7	4·0	27·5
IVDMD	0·935	0·398	0·611	0·104	17·1

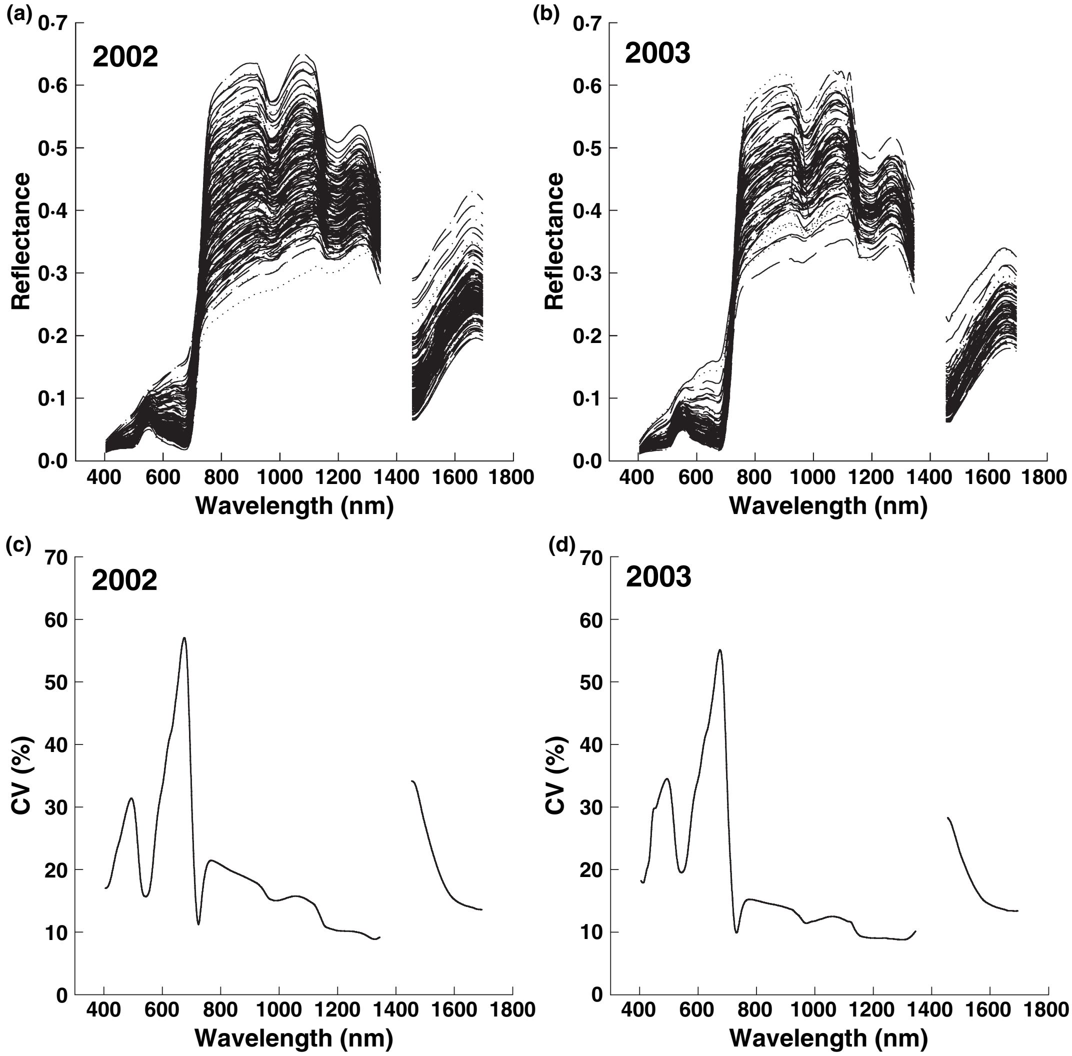

Figure 2 (a) and (b) Canopy reflectance at each wavelength measured by the portable spectroradiometer and (c) and (d) the coefficient of variation (CV) at each wavelength of the warm-season grass pastures in 2002 (n = 172) and 2003 (n = 92). Each reflectance spectra is the mean of three measurements at a given sampling site within each pasture. *Note:* the CV of canopy reflectance at 675 ± 5 nm is the greatest.

no increase in r^2 values for either N concentration or IVDMD of herbage (data not shown).

Validation of algorithm

Both N concentration and IVDMD of herbage in the validation data set were predicted using the equations in Table 2 and PLS regression models (not shown). The equations were visually evaluated through scatter plots of N concentration and IVDMD predicted by the respective equations vs. laboratory measurements (Figures 5 and 6).

Overall, N concentration of herbage was well predicted by canopy reflectance using all the statistical methods. The r^2 values ranged from 0·68 to 0·76; the RMSEP values ranged from 1·97 to 2·36 g N kg^{-1} DM; and the ME values ranged from 0·66 to 0·77 (Figure 5). Compared with the predictions of N concentration, the models for IVDMD yielded relatively low ME values (0·31–0·57), and most data points were not as clustered about the 1:1 lines (Figure 6) as was the case for N concentration. It is noted that the PLS regression involving all 120 wavebands in the model did not significantly increase ME values for predicting either N

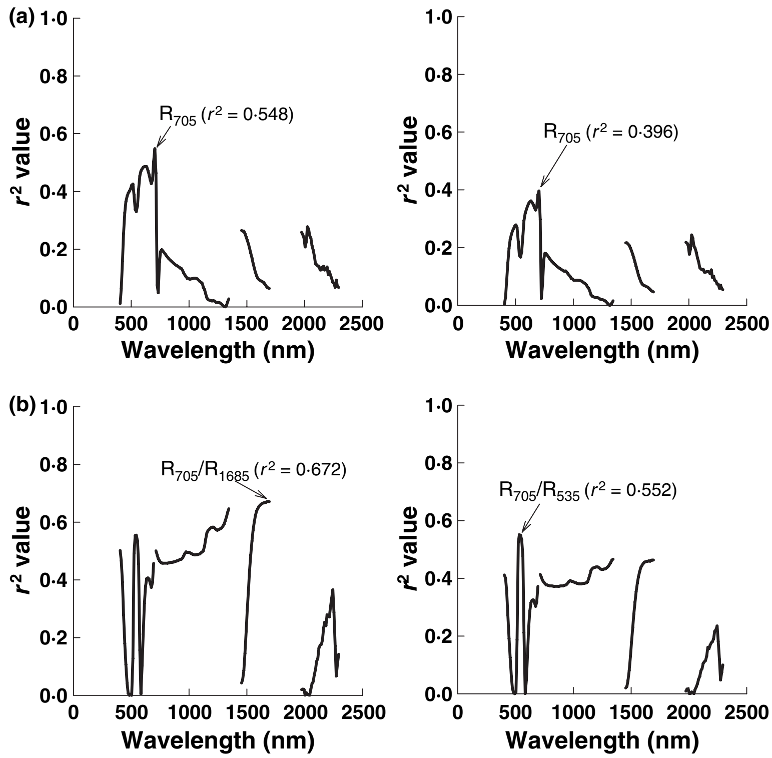

Figure 3 Coefficients of determination (r^2) vs. wavelength for the relationships of nitrogen concentration (N) and *in vitro* dry matter digestibility (IVDMD) of herbage with (a) canopy reflectance (R_i) and (b) reflectance ratios (R_{705}/R_i). The graphs on the left-hand side relate to N and those on the right-hand side relate to IVDMD. The r^2 values were based on a linear model of the calibration data set. Typical wavebands where the reflectance or reflectance ratios have the greatest r^2 (in parenthesis) are highlighted (n = 132).

Table 2 The best wavebands (±5 nm) selected from calculations of reflectance (R) ratio of the calibration data set for determining the linear relationships with nitrogen concentration (N) and *in vitro* dry matter digestibility (IVDMD) of herbage. The respective linear models and coefficients of determination (r^2) are also presented (n = 132).

Variable	Selected waveband (R, nm)	Equation	r^2
Simple linear regression of two-band ratio			
N	705 and 1685	$N = 31{\cdot}64 - 36{\cdot}91(R_1/R_2)$	0·672
IVDMD	705 and 535	$IVDMD = 1{\cdot}377 - 0{\cdot}447(R_1/R_2)$	0·552
Multiple regression of reflectance using MAXR*			
N	675, 705, 1495, 1535 and 1585	$N = 12{\cdot}38 + 232{\cdot}88(R_1) - 206{\cdot}23(R_2) + 321{\cdot}02(R_3) - 1066{\cdot}68(R_4) + 728{\cdot}98(R_5)$	0·801
IVDMD	505, 705, 1165, 1655 and 1695	$IVDMD = 0{\cdot}488 + 13{\cdot}916(R_1) - 6{\cdot}886(R_2) + 0{\cdot}934(R_3) - 24{\cdot}387(R_4) + 25{\cdot}034(R_5)$	0·649
Step-wise regression of band depth			
N	595, 605, 675, 685, 715, 965, 1025, 1035, 1175, 1225 and 1245	$N = 22{\cdot}65 - 566{\cdot}6\ (R_1) + 579{\cdot}3(R_2) + 551{\cdot}3\ (R_3) - 655{\cdot}0\ (R_4) + 110{\cdot}0(R_5) - 75{\cdot}2(R_6) - 210{\cdot}1(R_7) + 451{\cdot}0(R_8) + 187{\cdot}7(R_9) - 644{\cdot}0(R_{10}) + 362{\cdot}1(R_{11})$	0·814
IVDMD	465, 475, 485, 595, 605, 645, 715 and 975	$IVDMD = 0{\cdot}604 + 34{\cdot}034(R_1) - 42{\cdot}972(R_2) + 16{\cdot}547(R_3) - 19{\cdot}174(R_4) + 20{\cdot}224(R_5) - 3{\cdot}537(R_6) + 2{\cdot}903(R_7) - 3{\cdot}004(R_8)$	0·729

*Method of maximum r^2 improvement (MAXR).

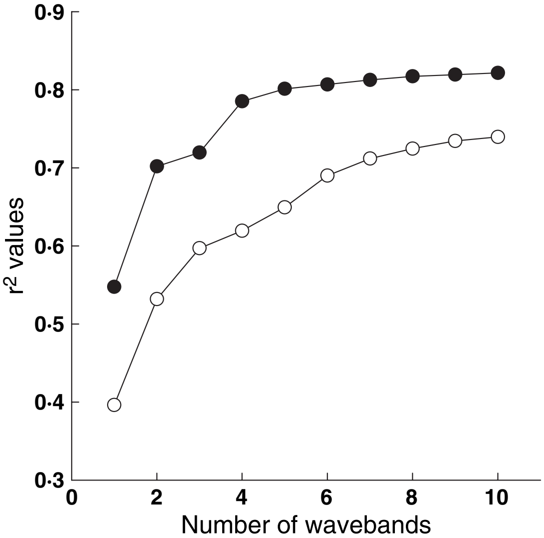

Figure 4 Relationship between r^2 values and the number of wavebands entering in the models during the multiple regression, using the method of maximum r^2 improvement, for nitrogen concentration (●) and *in vitro* dry matter digestibility (o) of herbage in the calibration data set (n = 132).

concentration or IVDMD of herbage compared with the simple linear regression of the two-band reflectance ratios and the regression using MAXR with five-band entrance.

Discussion

Canopy hyperspectral reflectance of warm-season grass pastures varied dramatically during the study period (Figure 2a and b). Changes in the canopy reflectance in the present study were primarily associated with chemical composition of the herbage because the canopies used in this study were generally closed, minimizing reflectance contributions from soil and understorey litter. Although the reflectance at 675 nm has the largest variation, i.e. the greatest CV, among 120 narrow wavebands (Figure 2b), it was not a canopy reflectance waveband associated closely with either N concentration or IVDMD of herbage (Figure 3).

Nitrogen concentration of herbage in grass pastures is an important indicator of nutritive value and changes considerably with growth stage, growth environment, plant genotype and management practices (Burzlaff, 1971; Taliaferro *et al.*, 2000; Starks *et al.*, 2006a). Spatial

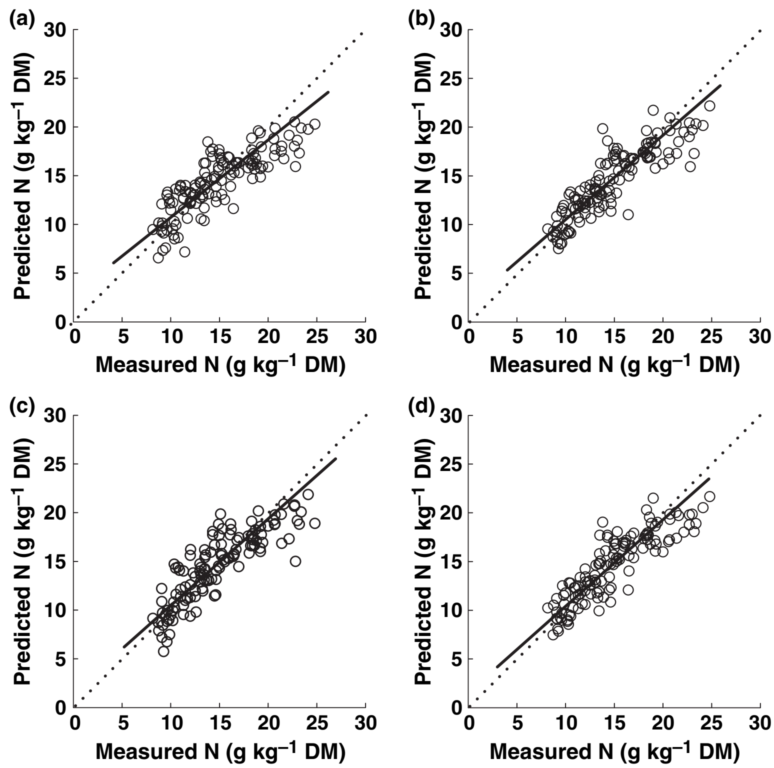

Figure 5 Relationships between laboratory-measured nitrogen (N) concentration of herbage in the test data set (n = 132) and estimated values based on (a) the reflectance ratio of R_{705}/R_{1685}, (b) multiple regression, using the method of maximum r^2 improvement, with five-waveband entrance, (c) continuum removal with step-wise regression with entrance of eleven wavebands and (d) partial least-squares regression with entrance of all 120 wavebands. The coefficients of determination (r^2), the root-mean-square error of prediction (RMSEP) and model efficiency (ME) are for (a) 0·68, 2·30 and 0·66 respectively; (b) 0·76, 1·97 and 0·75 respectively; (c) 0·72, 2·11 and 0·72 respectively and (d) 0·76, 1·97 and 0·77 respectively.

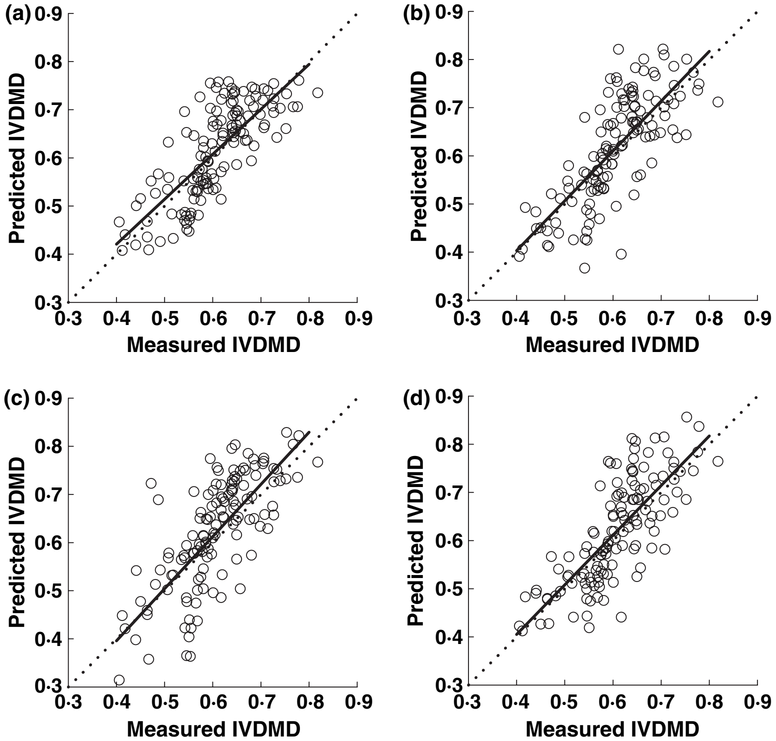

Figure 6 Relationships between laboratory-measured *in vitro* dry matter digestibility (IVDMD) of herbage and predictions using the validation data set (n = 132). Predicted values were based upon (a) the reflectance ratio of R_{705}/R_{535}, (b) multiple regression, using the method of maximum r^2 improvement, with five-waveband entrance, (c) continuum removal with step-wise regression with entrance of eleven wavebands and (d) partial least-squares regression with entrance of all 120 wavebands. The coefficients of determination (r^2), the root-mean-square error of prediction (RMSEP), and model efficiency (ME) are for (a) 0·58, 0·063 and 0·57 respectively; (b) 0·57, 0·074 and 0·42 respectively; (c) 0·53, 0·079 and 0·31 respectively and (d) 0·60, 0·068 and 0·48 respectively.

and temporal detection of N concentration in pastures can help ranchers better manage both pastures and grazing livestock. Moges *et al.* (2004) reported that during growth of winter wheat, N uptake by plants, i.e. accumulated N in plant vegetation, was closely related to the normalized difference vegetation index (NDVI) measured from canopy reflectance, but the relationship between the accumulated N and NDVI was non-linear. Starks *et al.* (2006a) found simple ratios of canopy reflectance in two broad wavebands (i.e. $R_{(blue)}/R_{(red)}$ or $R_{(NIR)}/R_{(red)}$) had better relationships with crude protein concentration of herbage as compared with NDVI. Studies on different plant species have indicated weak relationships between the concentration of N in plant tissues and reflectance in a single narrow waveband, and that two-waveband reflectance ratios of plant leaves correlate more closely with N concentration in leaves (Carter and Spiering, 2002; Read *et al.*, 2002; Zhao *et al.*, 2005a,b). The results of canopy reflectance in the present study are in agreement with these previous reports of the two-band reflectance ratios improving the relationship between N concentration of plant tissues and reflectance. More recently, Starks *et al.* (2006b) reported that a canopy reflectance ratio of R_{605}/R_{515} could be used to estimate crude protein concentration of herbage. While the present study indicated that the N concentration of herbage correlated closely with R_{605}/R_{515} (r^2 = 0·59, P < 0·001, data not shown), the ratio of R_{705}/R_{1685} was more closely related to N concentration of herbage (r^2 = 0·67, Table 2). These results further confirm that remote sensing of pasture, using canopy reflectance in two narrow wavebands, can be used to non-destructively estimate N or crude protein concentrations of the herbage of warm-season grass pastures during the growing season (Figure 5) and thus can help livestock managers make decisions concerning pasture and livestock management.

Literature addressing the relationship between IVDMD of herbage and canopy spectral reflectance is limited. The results of this study indicate the IVDMD of herbage of warm-season grass pastures is associated with canopy reflectance in some selected wavebands. Similar to N concentration of herbage, use of two-band reflectance ratios can improve the relationship between IVDMD and canopy reflectance as compared with simple reflectance in a single waveband (Figure 3). Although the r^2 value of the linear model developed from IVDMD of herbage and a selected canopy reflectance ratio is less than that of N concentration of herbage(Table 2), the predicted IVDMD values compared well to measured values (Figure 6a).

Several multiple regression methods, such as MAXR (Goel *et al.*, 2003), step-wise regression (Starks *et al.*, 2006a,b) and step-wise regression with band-depth analysis (Kokaly and Clark, 1999; Mutanga *et al.*, 2004), have been used to develop reflectance models for estimating the chemical constituents of plants. In the calibration data set in the present study, multiple regressions using the MAXR or step-wise method with band depth analysis increased r^2 values between canopy reflectance and N concentration and IVDMD of herbage (Table 2) compared with the models of two-band reflectance ratios. However, the multiple regression models only slightly (Figure 5b and c) or did not (Figure 6b and c) improve efficiency of prediction. These results further indicate that when canopy reflectance is used to predict N concentration and IVDMD of herbage, reflectance at most wavebands contribute little in assessment of N concentration and IVDMD and that a maximum of five to ten narrow wavebands is probably enough for estimation of nutritive value. It has been documented that PLS regression can be a useful tool when there is no practical need to limit the number of measured factors in the prediction equation (Tobias, 1995). In the present study, however, PLS regression did not increase accuracy of prediction for either N concentration or IVDMD of herbage. This result indicates that reflectance in a few wavebands contributes most in estimation of the nutritive value of herbage.

Conclusions

The results of this study demonstrate the potential of using data on canopy hyperspectral reflectance to estimate the N concentration and IVDMD of herbage of warm-season grass pastures. Several reflectance algorithms were developed: (i) using the simple linear regression of a two-waveband reflectance ratio and validated from a set of measurements taken in June and July; (ii) using multiple regression with MAXR and five waveband entries; (iii) using step-wise regression with band depth analysis and (iv) using PLS regression. Validation of these algorithms indicated that model prediction accuracy was similar among the different statistical methods. Compared with PLS regression, using all 120 wavebands, the multiple regression method using MAXR and incorporating five wavebands greatly reduced the number of selected wavebands needed to produce similar r^2, RMSEP and ME values. Simple reflectance ratios in two specific narrow wavebands may also be useful for timely and non-destructive prediction of N concentration and IVDMD of herbage in standing live pastures. Remote sensing of canopy reflectance holds promise as a useful tool for timely and non-destructive assessment of variables of nutritive value of herbage, such as N concentration and IVDMD, during pasture growth and grazing. It should be noted, however, that the general applicability of the algorithms developed above has not been tested at other locations or for other warm-season grasses.

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References

Adesogan A.T., Givens D.I. and Owens E. (2000) Measuring the chemical composition and nutritive value in forages. In: t'Mannetje L. and Jones R.M. (eds) *Field and laboratory methods for grassland and animal production research*, pp. 263–278. Wallingford, UK: CABI Publishing.

Ball D.M., Collins M., Lacefield G.D., Maitin N.P., Mertens D.A., Olson K.E., Putnam D.H., Undersander D.J. and Wolf M.W. (2001) *Understanding forage quality*. *American Farm Bureau Federation, Publication 1-01*. Park Ridge, IL, USA: American Farm Bureau Federation.

Burzlaff D.F. (1971) Seasonal variations of the *in vitro* dry matter digestibilityof three sandhill grasses. *Journal of Range Management*, **20**, 60–63.

Campbell J.B. (1996) *Introduction to remote sensing*, 2nd edn. London, UK: The Guildford Press.

Carter G.A. and Spiering B.A. (2002) Optical properties of intact leaves for estimating chlorophyll concentration. *Journal of Environmental Quality*, **31**, 1424–1432.

Chappelle E.W., Kim M.S. and McMurtrey J.E. III (1992) Ratio analysis of reflectance spectra (RARS): an algorithm for the remote estimation of the concentrations of chlorophyll *a*, and chlorophyll *b*, and carotenoids in soybean leaves. *Remote Sensing of Environment*, **39**, 239–247.

Curran P.J., Dungan J.L., Macler B.A., Plummer S.E. and Peterson D.L. (1992) Reflectance spectroscopy of fresh whole leaves for the estimation of chemical concentration. *Remote Sensing of Environment*, **39**, 153–166.

Daughtry C.H.T., Walthall C.L., Kim M.S., de Colstoun E.B. and McMurtrey J.E. III (2000) Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. *Remote Sensing of Environment*, **74**, 229–239.

Everitt J.H., Richardson A.J. and Gausman H.W. (1985) Leaf reflectance-nitrogen-chlorophyll relations in buffelgrass. *Photogrammetric Engineering and Remote Sensing*, **51**, 463–466.

Fales S.L. and Fritz J.O. (2007) Factors affecting forage quality. In: Barnes R.F., Miller D.A. and Nelson C.J. (eds) *Forages, volume 2: the science of grassland agriculture*, 5th edn, pp. 569–580. Ames, IA, USA: Blackwell Publishers.

Goel P.K., Prasher S.O., Landry J.A., Patel R.M., Viau A.A. and Miller J.R. (2003) Estimation of crop biophysical parameters through airborne and field hyperspectral remote sensing. *Transactions of the American Society of Agricultural Engineers*, **46**, 1235–1246.

Goering H.K. and Van Soest P.J. (1970) *Forage fibre analysis (apparatus, reagents, procedures and some applications). Agricultural handbook, No. 379*. Washington, DC, USA: Agricultural Research Service, US Department of Agriculture.

Kamalak A., Filbo J.M.P., Canbolat O., Gurbuz Y., Ozay O. and Ozkan C.O. (2004) Chemical composition and its relationship to in vitro dry matter digestibility of several tannin-containing trees and shrub leaves. *Livestock Research for Rural Development*, **16**, 7. Available at: http://www.cipav.org.co/lrrd/lrrd16/4/kamal6027.htm (accessed 11 October 2007).

Kokaly R.F. and Clark R.N. (1999) Spectroscopic determination of leaf biochemistry using band-depth analysis of absorption features and step-wise multiple linear regression. *Remote Sensing of Environment*, **67**, 267–287.

Lamb D.W., Steyn-Ross M., Schaare P., Hanna M.M., Silvester W. and Steyn-Coss A. (2002) Estimating leaf nitrogen concentration in ryegrass (*Lolium* spp.) pasture using the chlorophyll red-edge: theoretical modeling and experimental observations. *International Journal of Remote Sensing*, **23**, 3619–3648.

Moges S.M., Raun W.R., Mullen R.W., Freeman K.W., Johnson G.V. and Solie J.B. (2004) Evaluation of green, red, and near infrared bands for predicting winter wheat biomass, nitrogen uptake, and final grain yield. *Journal of Plant Nutrition*, **27**, 1431–1441.

Mutanga O., Skidmore A.K. and Prins H.H.T. (2004) Predicting *in situ* pasture quality in the Kruger National Park, South Africa, using continuum-removed absorption features. *Remote Sensing of Environment*, **89**, 393–408.

Nash J.E. and Sutcliffe J.V. (1970) River flow forecasting through conceptual models. Part 1. A discussion of principles. *Journal of Hydrology*, **10**, 282–290.

Peñuelas J. and Filella I. (1998) Visible and near-infrared reflectance techniques for diagnosing plant physiological status. *Trends of Plant Science*, **3**, 151–156.

Peñuelas J. and Inoue Y. (2000) Reflectance assessment of canopy CO_2 uptake. *International Journal of Remote Sensing*, **21**, 3353–3356.

Read J.J., Tarpley L., McKinion J.M. and Reddy K.R. (2002) Narrow-waveband reflectance ratios for remote estimation of nitrogen status in cotton. *Journal of Environmental Quality*, **31**, 1442–1452.

Richardson A.J., Everitt J.H. and Gausman H.W. (1983) Radiometric estimation of biomass and nitrogen content of Alicia grass. *Remote Sensing of Environment*, **13**, 179–184.

Riday H. and Brummer E.C. (2006) Relationships among biomass yield components within and between subspecies of alfalfa. Medicago Genetic Reports, Vol. 4, 7pp. Available at: http://www.medicago-reports.org/pdfs/Riday_Brummer_falcata.pdf (accessed 11 October 2007).

SAS (1997) *SAS user guide: statistics*. Cary, NC, USA: SAS Institute Inc.

Serrano L., Peñuelas J. and Ustin S.L. (2002) Remote sensing of nitrogen and lignin in Mediterranean vegetation from AVIRIS data: decomposing biochemical from structural signals. *Remote Sensing of Environment*, **81**, 355–364.

Sims D.A. and Gamon J.A. (2002) Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment*, **81**, 337–354.

Starks P.J., Coleman S.W. and Phillips W.A. (2004) Determination of forage chemical composition using remote sensing. *Journal of Range Management*, **57**, 635–640.

Starks P.J., Zhao D., Phillips W.A. and Coleman S.W. (2006a) Herbage mass, nutritive value and canopy spectral reflectance of bermudagrass pastures. *Grass and Forage Science*, **61**, 101–111.

Starks P.J., Zhao D., Phillips W.A. and Coleman S.W. (2006b) Development of canopy reflectance algorithms for real-time prediction of bermudagrass pasture biomass and nutritive values. *Crop Science*, **46**, 927–934.

Taliaferro C.M., Williams G.L., Pickard T.G., Hooper D.W., Coleman S.W. and Phillips W.A. (2000) *Performance of forage bermudagrass varieties in Oklahoma, 1995–1999*. Production technology-crops PT-2000-8. Oklahoma, USA: Cooperative Extension Service, Oklahoma State University.

Tobias R.D. (1995) An introduction to partial least squares regression, Available at: http://support.sas.com/techsup/technote/ts509.pdf (accessed 11 October 2007).

Yoder B.J. and Pettigrew-Crosby R.E. (1995) Predicting nitrogen and chlorophyll content and concentrations from reflectance spectra (400–2500 nm) at leaf and canopy scales. *Remote Sensing of Environment*, **53**, 199–211.

Yu C.H. (2000) An overview of remedial tools for collinearity in SAS, *Proceedings of 2000 western users of SAS software conference, September 17–19, 2000*, Western Users of SAS Software, Inc., Scottsdale, AZ, 196–201.

Zhao D., Reddy K.R., Kakani V.G., Read J.J. and Koti S. (2005a) Selection of optimum reflectance ratios for estimating leaf nitrogen and chlorophyll concentrations of field-grown cotton. *Agronomy Journal*, **97**, 89–98.

Zhao D., Reddy K.R., Kakani V.G. and Reddy V.R. (2005b) Nitrogen deficiency effects on plant growth, leaf photosynthesis and hyperspectral reflectance properties of sorghum. *European Journal of Agronomy*, **22**, 391–403.

